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**Comparative study of BAP1 and CD147
expression in diagnosis of malignant
mesothelioma**

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Welcome letter from Editor-in-Chief



Welcome to the Int J Cancer and Biomedical Research (IJCBR)!

It is with great pleasure that I write this editorial to welcome you to the IJCBR. This journal provides a platform for publication of original and reviews research articles, short communications, letter to editor, thesis abstract, conference report, and case studies. These types of publication are directed at the interface of the fields of cancer and biomedical research.

The IJCBR relies on a distinguished expert of the Advisory and Editorial Board Members from the top international league covering in depth the related topics. They timely review all manuscripts and maintain highest standards of quality and scientific methodology and ethical concepts. Meanwhile, we take all possible means to keep the time of the publication process as short as possible.

I take this chance to welcome your contributions to the IJCBR and have every expectation that it will soon become one of the most respected journals in both the fields of cancer and biomedical research.

A handwritten signature in blue ink that reads "Mohamed L. Salem". The signature is fluid and cursive.

Mohamed L. Salem,

Editor in Chief

Comparative study of BAP1 and CD147 expression in the diagnosis of malignant mesothelioma

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ABSTRACT

Background: Malignant mesothelioma (MM) is a lethal tumor of serosal surfaces. Its differentiation from reactive mesothelial hyperplasia is mandatory, and may be problematic in many situations. Thus, the application of a targeted panel of specific markers permits proper diagnosis in the majority of cases. **Aim:** This study aimed to determine the potential use of BRCA associated protein-1 (BAP1) and CD147 antibodies to differentiate between MM and reactive mesothelial hyperplasia. **Materials and Methods:** The current work was carried out on 120 cases (56 reactive mesothelial hyperplasia and 64 malignant mesothelioma cases), retrieved as 70 cell blocks and 50 tissue samples. Immunohistochemical staining, using calretinin (to confirm the mesothelial lineage), BAP1, and CD147 antibodies, was performed for each case. **Results:** Lost nuclear BAP1 expression was detected in 45.3% of the mesotheliomas versus 19.6% of reactive mesothelial hyperplasia cases. Positive membranous CD147 expression was found in 84.4% of mesothelioma cases versus 8.9% of reactive mesothelial hyperplasia cases. BAP1 showed 44.6% sensitivity and 80% specificity, while CD147 showed 84.3% sensitivity and 91% specificity in the diagnosis of malignant mesothelioma. **Conclusions:** The loss of BAP1 expression and positive CD147 expression could be of diagnostic value for malignant mesothelioma and can exclude the diagnosis of reactive mesothelial proliferations in both biopsy and cytology specimens. CD147 exhibits higher sensitivity and specificity than BAP1.

Keywords: Malignant mesothelioma; reactive mesothelial hyperplasia; BAP1; CD147

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INTRODUCTION

Malignant mesothelioma (MM) is an aggressive tumor of mesothelial origin, involving mainly the pleura; with an increasing incidence worldwide, due to asbestos exposure. MM is related to poor survival, particularly for the pleomorphic and sarcomatoid/desmoplastic variants (Robinson and Lake, 2005).

Early appropriate diagnosis of MM may improve patient outcomes. However, it may be difficult to differentiate between a wide spectrum of reactive and neoplastic mesothelial proliferations, depending only on routine histologic examination. MM cells may appear innocent, while hyperplastic mesothelial cells may exhibit various degrees of cellular atypia; especially, in cytological specimens. Also, it's difficult to identify the invasive component of MM in small biopsies. Superficial entrapment of

mesothelial cells by organized effusion is common in reactive mesothelial proliferations, which may be confused with malignant invasion (Minato et al., 2014). Thus, many studies investigated the role of novel biomarkers in an attempt to differentiate between MM and reactive mesothelial cells. However, the results of those studies were contradictory (Chapel et al., 2020).

BRCA associated protein-1 (BAP1) is a tumor suppressor gene, located at 3p21. BAP1 acts as a deubiquitinating enzyme, involved in the removal of ubiquitin from proteins (McGregor et al., 2015 and Boffetta et al., 2018). Nuclear-localized, ubiquitin carboxy-terminal hydrolase proteins play an important role in the ubiquitin-dependent proteasome pathway, which is responsible for protein degradation. This process is necessary for removal of abnormally folded or damaged proteins, regulation of gene

transcription, and DNA damage repair responses. Disruption of ubiquitin-dependent proteasome pathway is related to several cancer types and could be a potential target for anti-cancer therapies (Cigognetti et al., 2015).

BAP1 also modulates calcium-induced apoptosis, and so, its mutation ends in accumulation of DNA-damaged cells with a high susceptibility to develop malignancy, as seen in malignant mesothelioma, uveal tract and cutaneous melanoma (Carbone et al., 2012; Baumann et al., 2015 and Bononi *et al*, 2017). Mutation of BAP1, identified as undetectable nuclear immunoreactivity, has been suggested as a potential marker to identify MM, particularly in small biopsies (Erber et al., 2020). CD147 (or Extracellular matrix metalloproteinase inducer “EMMPRIN”), is a cell surface glycoprotein and a member of immunoglobulin superfamily (Ig SF) [that are involved in the recognition, binding, or adhesion processes of cells]. CD147 plays a role in intercellular communication, involved in many immune-related functions, differentiation, and development (Dai et al., 2013). It's the chaperone of monocarboxylate transporters (MCTs), which mediate the transmembrane co-transport of lactate, thereby, regulating pH of the tumor microenvironment.

CD147 also acts as a key marker of cell invasion and metastasis in many cancers, via production of numerous matrix metalloproteinases. Its expression is related to advanced stage, higher grade, lymphovascular invasion, and decreased overall survival. CD147 dampens host immune defenses and favors chemoresistance (Afonso et al., 2009). So, CD147 silencing could be a target for therapeutic strategies in many tumors, including MM (Pinheiro et al., 2012).

In the current study, we aimed to investigate the potential diagnostic role of BAP1 and CD147 in differentiating MM and reactive mesothelial hyperplasia.

MATERIALS AND METHODS

This retrospective study was carried out on 120 formalin-fixed paraffin-embedded (FFPE) blocks (56 reactive mesothelial hyperplasia and 64 malignant mesothelioma cases). Cases were

retrieved as 70 cell blocks of cytological preparations and 50 tissue biopsy samples.

These cases were selected from the archives of the Pathology Department, Faculty of Medicine, Tanta University, during the period of the study from March 2019 to June 2020, depending on quality of the blocks and presence of full clinical data. Approval from the research ethics committee, Faculty of Medicine, Tanta University, was obtained before conducting the study.

All cases were stained by routine H&E staining, for proper re-evaluation and by calretinin immunohistochemical staining (clone H-5: sc-365956, Santa Cruz Biotechnology, INC, USA, dilution 1:100), to confirm the mesothelial origin of the selected cases. Sections of the obtained blocks (5 µm thick), on positively charged slides, were left to dry for 30 minutes at 37°C. Deparaffinization and antigen retrieval were performed in a Dako PT Link unit. Both high and low pH EnVision™ FLEX Target Retrieval Solutions were used at 97°C for 20 min. Dako automated immune-stainer (Link 48) was used for immunostaining.

Anti-BAP1 antibody, a mouse monoclonal antibody (clone C-4, sc-28383, Santa Cruz Biotechnology, INC, USA) and anti-CD147 antibody, a mouse monoclonal antibody (clone 8D6: sc-21746, Santa Cruz Biotechnology, INC, USA) were used as primary antibodies. The slides were incubated with the primary antibodies for 30 minutes, following treatment with a peroxidase-blocking reagent for 5 minutes; with subsequent addition of horseradish peroxidase (HRP) reagent for 20 min and diaminobenzidine (DAB) chromogen solution for 10 minutes. Hematoxylin was applied for counterstaining.

Assessment of BAP1 immunohistochemical results

Only nuclear immunoreactivity, at any percentage, was considered positive (although some cases showed finely granular cytoplasmic reaction; interpreted as negative reaction according to Chapel et al., 2020). Positive internal control for BAP1 was observed in normal/reactive mesothelial cells and stromal fibroblastic cells, as nuclear brownish staining

(inflammatory cells and vascular endothelium may show non-specific staining, as well).

Assessment of CD147 immunohistochemical results

Considering both the intensity and extent of CD147 expression; the final score (ranging from 0-6) was determined. The intensity of staining was scored as follows: 0: negative; 1: weak; 2: intermediate; and 3: strong. CD147 staining extent was scored as follows: 0: 0% of immunoreactive cells; 1: <5% of immunoreactive cells; 2: 5–50% of immunoreactive cells; and 3: >50% of immunoreactive cells. Final scores ≥ 3 were considered positive (Pinheiro et al., 2012). Sections of urothelial carcinoma were used as a positive control for CD147, in which CD147 expressed as brownish membranous staining in the tumor cells.

Statistical analysis

The collected data were statistically analyzed using the SPSS software statistical computer package (version 23, Armonk, NY: IBM Corp). Data were expressed in terms of frequencies and percentages for categorical variables; range, median, and mean \pm standard deviation (SD) for continuous variables.

To confirm the diagnostic role of BAP1 and CD147; the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for each marker were calculated as follows:

Sensitivity: True positive / (True positive + False negative) $\times 100$

Specificity: True negative / (True negative + False positive) $\times 100$

PPV: True positive / (True positive + False positive) $\times 100$

NPV: True negative / (True negative + False negative) $\times 100$

Accuracy: (True positive + True negative) / (True positive + True negative + False positive + False negative) $\times 100$

Statistical relation between BAP1 and CD147 was performed using McNemar test. P-value of < 0.05 was considered statistically significant.

RESULTS

Clinicopathological data

This study included 120 mesothelial specimens; whose ages ranged from 40 to 75 years (median 57.5 years). Regarding the sex of the studied cases, 101 cases (84.2%) were males and 19 cases (15.8%) were females. One hundred cases (83.3%) were pleural in origin and the remaining 20 cases (16.7%) were peritoneal. Out of the studied cases, 64 cases (53.3%) were malignant mesotheliomas (including 56 cases epithelioid and 8 cases Sarcomatoid mesothelioma) and 56 cases (46.7%) were reactive mesothelial hyperplasia. Clinicopathological characteristics of studied cases are summarized in Table 1.

Immunohistochemical staining results of BAP1

Lost nuclear BAP1 expression was detected in 33.3% of all the studied cases. Among the cases of mesothelioma; 45.3% showed negative BAP1 expression: 46.4% of the epithelioid mesothelioma cases and 37.5% of the sarcomatoid mesotheliomas showed lost nuclear BAP1 staining. Regarding reactive mesothelial hyperplasia cases; 19.6% showed lost nuclear BAP1 expression (Table 2 and Figure 1).

Immunohistochemical staining results of CD147

Positive CD147 expression was demonstrated as membranous staining in 49.2% of our cases. Among mesothelioma cases; 84.4% showed positive CD147 expression (85.7% of the epithelioid mesothelioma cases and 75% of the sarcomatoid mesotheliomas were CD147 positive). As regard to reactive mesothelial hyperplasia cases; only 8.9% showed CD147 positivity (Table 3 and Figure 2). The sensitivity, specificity, PPV, NPV, and accuracy of BAP1 and CD147 in the diagnosis of malignant mesothelioma are summarized in Table 4. CD147 showed higher sensitivity, specificity, PPV, NPV, and accuracy than BAP1.

Table 5 shows a statistically significant negative relation between BAP1 expression and CD147 immunoreactivity in the studied mesothelial cases (P = 0.026). As 75% of cases with lost BAP1 expression, showed positive CD147 staining; while 63.7% of cases with retained BAP1 expression, showed CD147 negative staining.

Table 1. The clinicopathological characteristics of the studied cases

Clinicopathological characteristics	Cases (No.)	%
Age in years \pm SD (range)	57.5 \pm 8.605 (40-75)	
Sex		
Male	101	84.2%
Female	19	15.8%
Specimen		
Cell blocks (Cytology)	70	58.3%
Tissue biopsy	50	41.7%
Location		
Pleural	100	83.3%
Peritoneal	20	16.7%
Histopathological types		
Mesothelioma (total)	64	53.3%
Epithelioid	56	46.7%
Sarcomatoid	8	6.6%
Reactive mesothelial hyperplasia	56	46.7%

Table 2. BRCA associated protein-1 (BAP1) expression in the studied cases

BAP1 expression	Cases (No.)	Lost expression N (%)	Retained expression N (%)
Total	120	40 (33.3)	80 (66.7)
Histopathological types			
Mesothelioma	64	29 (45.3)	35 (54.7)
Epithelioid	56	26 (46.4)	30 (53.6)
Sarcomatoid	8	3 (37.5)	5 (62.5)
Reactive mesothelial hyperplasia	56	11 (19.6)	45 (80.4)

Table 3. CD147 expression in the studied cases

CD147 expression	Cases (No.)	Positive expression N (%)	Negative expression N (%)
Total	120	59 (49.2)	61 (50.8)
Histopathological types			
Mesothelioma	64	54 (84.4)	10 (15.6)
Epithelioid	56	48 (85.7)	8 (14.3)
Sarcomatoid	8	6 (75)	2 (25)
Reactive mesothelial hyperplasia	56	5 (8.9)	51 (91.1)

Table 4. The sensitivity, specificity, PPV, NPV, and accuracy of BRCA associated protein-1 (BAP1) and CD147 in the diagnosis of malignant mesothelioma

	Sensitivity (%)	Specificity (%)	PPV* (%)	NPV** (%)	Accuracy (%)
BAP1	44.6	80	72.5	56.3	61.7
CD147	84.3	91	91.5	83.6	87.5

*PPV, Positive predictive value, **NPV, Negative predictive value

Table 5. The relation between BRCA associated protein-1 (BAP1) and CD147 expression in the studied cases

BAP1 (n=120)	CD147 (n=120)	
	Positive (n=59) N (%)	Negative (n=61) N (%)
Retained (n=80)	29 (36.3)	51 (63.7)
Lost (n=40)	30 (75)	10 (25)
P	0.026*	

*Statistically significant (P < 0.05)

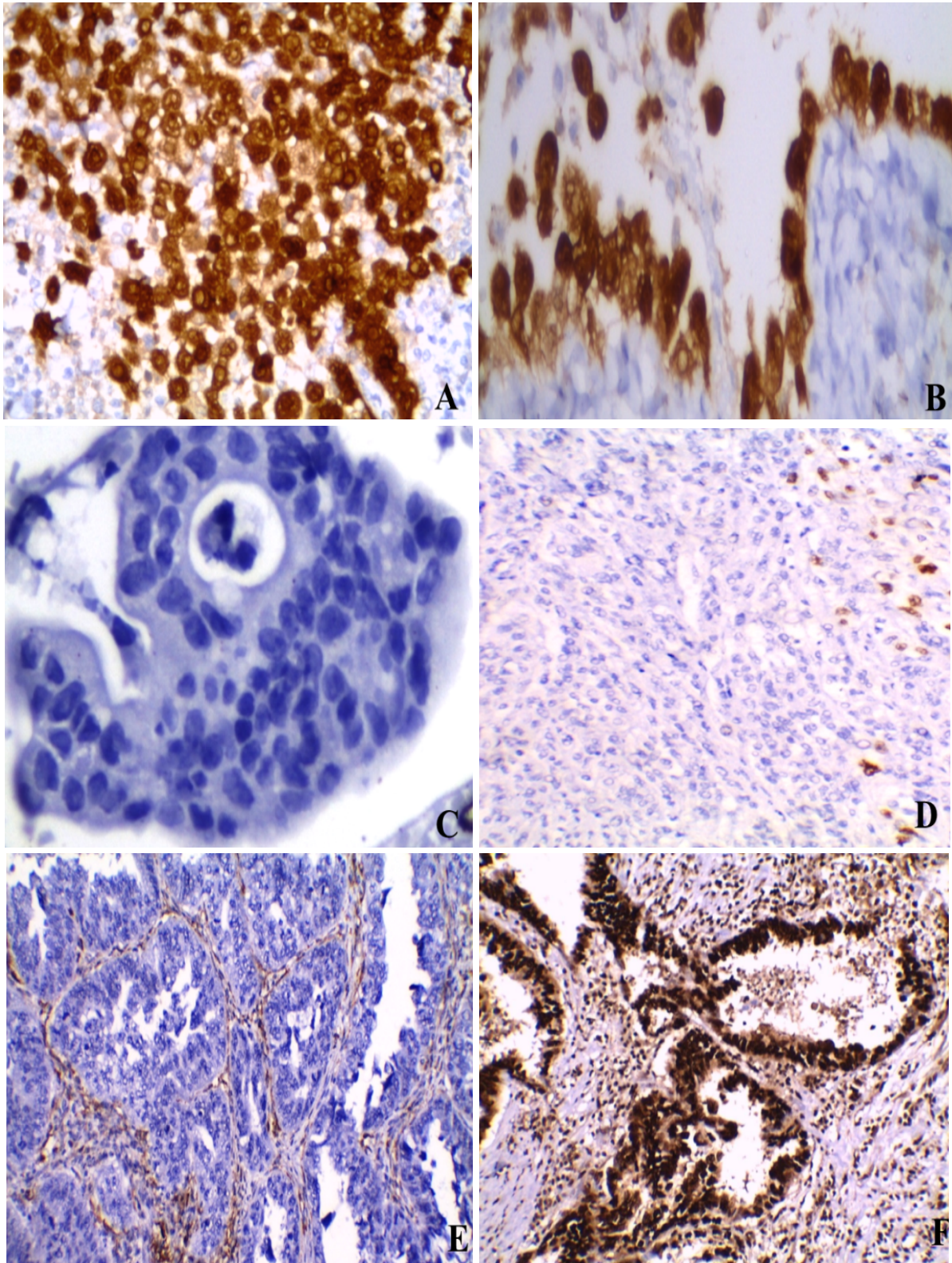


Figure 1. Reactive mesothelial hyperplasia showing retained nuclear BRCA associated protein-1 (BAP1) expression on cytological specimen (cell block) (x400) (A), and tissue biopsy specimen (x400) (B), Malignant mesothelioma cells showing negative nuclear BAP1 expression on cytological specimen (cell block) (x400) (C), and tissue biopsy specimen (x200) (D), Malignant mesothelioma (epithelioid type) showing negative BAP1 expression on tissue biopsy specimen, with surrounding BAP1 positive stromal cells (x200) (E), Malignant mesothelioma (epithelioid type) showing positive BAP1 expression on tissue biopsy specimen, with surrounding BAP1 positive stromal and inflammatory cells (x200) (F).

DISCUSSION

Malignant mesothelioma (MM) is a rare lethal tumor of the serosal surfaces. Its prognosis is poor, with a median survival time rarely exceeding 12 months (Robinson and Lake, 2005 and Ray and Kindler, 2009).

Accurate pathologic diagnosis of MM leads to early proper therapy, including surgery, and can improve the patients' outcomes. Distinction between malignant mesothelioma and benign mesothelial proliferations is challenging in many conditions; especially in tiny specimens, where stromal invasion cannot be assessed or cytological samples, where tumor architecture is difficult to evaluate (Monaco et al., 2018 and Chapel et al., 2020).

To differentiate, many immunohistochemical markers have been developed, but their significance remains controversial, with marked variation in their sensitivity and specificity among various reports.

In this study, we evaluated the diagnostic role of BAP1 and CD147 immunohistochemical expression in differentiating malignant mesothelioma and reactive mesothelial hyperplasia. Regarding BAP1 expression, 45.3% of the studied mesothelioma cases showed lost BAP1 expression, in contrast to cases of reactive mesothelial hyperplasia, which showed loss of BAP1 expression among only 19.6%.

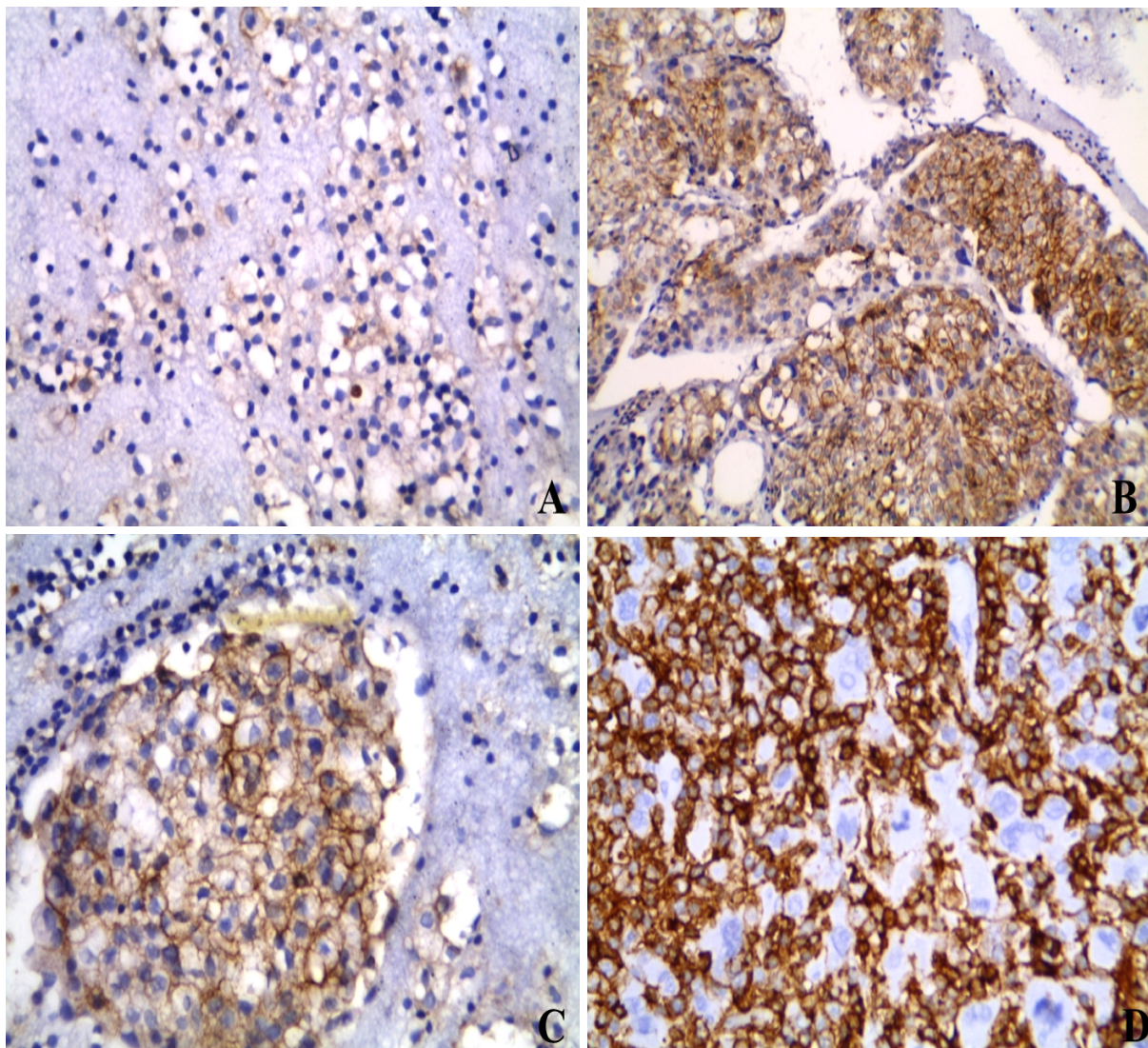


Figure 2. Reactive mesothelial hyperplasia showing negative CD147 expression on cytological preparation (cell block) (x400) (A), Malignant mesothelioma cells showing positive CD147 expression on cytological specimen (cell block) (x200) (B), Malignant mesothelioma cells showing positive CD147 expression, compared to negative CD147 expression of the surrounding reactive mesothelial cells on cytological specimen (cell block) (x400) (C), Malignant mesothelioma (epithelioid type) showing positive CD147 expression on tissue biopsy specimen (x400) (D).

These results matched those of Erber et al., 2020, who observed lost BAP1 expression among 56.4% of their MM cases. Kinoshita et al., 2018^(a) also studied BAP1 to differentiate sarcomatoid mesothelioma and fibrosing pleuritis and found that BAP1 showed 100% specificity (using both IHC and FISH technology). According to other studies, BAP1 loss is also 100% specific for malignancy in mesothelial proliferations (Hida et al., 2017; Berg et al., 2018; Hatem et al., 2019, and Yoshimura et al., 2019). The lower BAP1 specificity in our study, compared with the previous studies, maybe due to lack of genomic analysis, or different ways to interpret BAP1 immunostaining.

According to our results, BAP1 was relatively insensitive marker (low sensitivity of 44.6%) in detecting malignant mesothelioma. Our results matched those of Nasu et al., 2015; Hwang et al., 2016^(a); Righi et al., 2016; Hida et al., 2017; Yoshimura et al., 2017; Chou et al., 2018; Kinoshita et al., 2018^(b), and Yoshimura et al., 2019, who all reported low BAP1 sensitivity, ranging from 50–65%.

The PPV of BAP1 in the diagnosis of MM in this study was 72.5%; however according to both Cozzi et al., 2018 and Cigognetti et al., 2015, the PPV of BAP1 reached 100%. The NPV value of BAP1 in the diagnosis of MM in the current study was 56.3%. Slightly lower results were detected by Cozziet al., 2018, who reported 49.3% NPV in their work. Cigognetti et al., 2015 stated that the NPV of BAP1 in their study was 90%. Hwang et al., 2016b reported that BAP1 and p16 are not lost in all MM, using FISH analysis, and even when used together, lost expression couldn't confirm the malignant nature of the lesions. Husain et al., 2018 stated that loss of nuclear BAP1 expression is a malignancy indicator, but it does exclude a benign diagnosis. Accordingly, interpretation of BAP1 expression should be done with caution, especially when considering a primary diagnosis of malignant mesothelioma and it is better confirmed by another marker.

In our work, lost nuclear BAP1 expression was detected in 46.4% versus 37.5% of the epithelioid and sarcomatoid mesotheliomas, respectively. These results were agreed with

Erber et al., 2020, who stated that 55.4% of epithelioid and 41.7% of sarcomatoid MM were

BAP1-deficient. Bott et al., 2011, did not find a relation between BAP1 (by genomic analysis) and histologic mesothelioma variants. On the contrary, Cigognetti et al., 2015, found a striking difference in BAP1 loss among epithelioid and sarcomatoid mesothelioma (69% versus 15%). Shinozaki-Ushiku et al., 2017 reported BAP1 loss in 61% of epithelioid versus 0% loss among sarcomatoid variants of MM. Owing to low sensitivity of BAP1 in the diagnosis of malignant mesothelioma, we studied CD147 expression among benign and malignant mesothelial proliferations, to determine if it has better diagnostic utility. Regarding CD147 expression in this study, 84.4% of the studied mesothelioma cases showed positive CD147 expression, versus 8.9% of reactive mesothelial hyperplasia cases. CD147 showed 84.3% sensitivity and 91% specificity in the diagnosis of malignant mesothelioma (both were higher than BAP1).

Only few studies have investigated CD147 expression among reactive mesothelial cells and mesothelioma cases. The main was of Pinheiro et al., 2012, who stated that CD147 can distinguish these two proliferations and that CD147 could be a target for MM targeted therapy. Their sample size was small (20 cases), and they found that CD147 was significantly expressed in 90% (8/9) of MM vs. 9% (1/11) for mesothelial reactive cells, with 88.8% sensitivity and 90.9% specificity. Later on, Paintal et al., 2013, recommended further study of CD147 expression among mesothelial cases to determine its diagnostic validity and explore its potential therapeutic use.

CONCLUSION

Positive CD147 expression and lost BAP1 expression may be diagnostic of malignant mesothelioma and can exclude the diagnosis of reactive mesothelial proliferations. CD147 exhibits higher sensitivity, specificity, PPV, NPV and accuracy than BAP1. Further studies, using IHC and gene analysis, are required to confirm the validity of the current results in diagnosis of benign and malignant mesothelial proliferations, and to explore the potential therapeutic role of BAP1 and CD147 in MM.

CONFLICTS OF INTEREST

All authors declared no conflict of interest.

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Egyptian Association for Cancer Research (EACR)

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EACR is an NGO society that was declared by the Ministry of Social Solidarity (Egypt) No. 1938 in 19/11/2014 based on the initiative of Prof. Mohamed Labib Salem, the current Chairman of EACR. EACR aims primarily to assist researchers, in particular young researchers in the field of cancer research through workshops, seminars and conferences. Its first international annual conference entitled "Anti-Cancer Drug Discovery" was successfully organized in April 2019 (<http://acdd.tanta.edu.eg>). Additionally, EACR aims to raise the awareness of the society about the importance of scientific research in the field of cancer research in prediction, early diagnosis and treatment of cancer. EACR is also keen to outreach the scientific community with periodicals and news on cancer research including peer-reviewed scientific journals for the publication of cutting-edge research. The official scientific journal of EACR is "International Journal of Cancer and biomedical Research (IJCBR: <https://jcbjournals.ekb.eg>) was successfully issued in 2017 and has been sponsored by the Egyptian Knowledge Bank (EKB: www.ekb.eg).

EACR Chairman,**Prof. Mohamed Labib Salem, PhD**

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GUIDE FOR AUTHORS

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